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ORIGINAL ARTICLE

Prenatal maternal immune activation causes epigenetic differences in adolescent mouse brain

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Epigenetic processes such as DNA methylation have been implicated in the pathophysiology of neurodevelopmental disorders including schizophrenia and autism. Epigenetic changes can be induced by environmental exposures such as inflammation. Here we tested the hypothesis that prenatal inflammation, a recognized risk factor for schizophrenia and related neurodevelopmental conditions, alters DNA methylation in key brain regions linked to schizophrenia, namely the dopamine rich striatum and endocrine regulatory centre, the hypothalamus. DNA methylation across highly repetitive elements (long interspersed element 1 (LINE1) and intracisternal A-particles (IAPs)) were used to proxy global DNA methylation. We also investigated the *Mecp2* gene because it regulates transcription of LINE1 and has a known association with neurodevelopmental disorders. Brain tissue was harvested from 6 week old offspring of mice exposed to the viral analog PolyI:C or saline on gestation day 9. We used Sequenom EpiTYPER assay to quantitatively analyze differences in DNA methylation at IAPs, LINE1 elements and the promoter region of *Mecp2*. In the hypothalamus, prenatal exposure to PolyI:C caused significant global DNA hypomethylation ($t = 2.44$, $P = 0.019$, PolyI:C mean 69.67%, saline mean 70.19%), especially in females, and significant hypomethylation of the promoter region of *Mecp2*, ($t = 3.32$, $P = 0.002$; PolyI:C mean 26.57%, saline mean 34.63%). IAP methylation was unaltered. DNA methylation in the striatum was not significantly altered. This study provides the first experimental evidence that exposure to inflammation during prenatal life is associated with epigenetic changes, including *Mecp2* promoter hypomethylation. This suggests that environmental and genetic risk factors associated with neurodevelopmental disorders may act upon similar pathways. This is important because epigenetic changes are potentially modifiable and their investigation may open new avenues for treatment.

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INTRODUCTION

Epigenetic processes are crucial for the regulation of genomic function and development.[1] For example, DNA methylation is implicated in X-inactivation, genomic imprinting control and remote silencing of genomic regions.[2] Tissue-specific epigenetic modifications through CpG methylation are thought to modulate brain development, circadian rhythm and disease status.[3–5] Consistent with this, epigenetic anomalies have been linked to complex neurodevelopmental disorders including schizophrenia and autism spectrum disorders.[6,7]

In early brain development, environmental exposures such as inflammation,[8] diet,[9,10] toxins and contaminants[11] disrupt developmental trajectories. For example, prenatal exposure to inflammation has been implicated in the etiology of schizophrenia[12] autism[13] and bipolar disorder.[14] Direct evidence supporting a role for maternal immune activation (MIA) during prenatal life in neurodevelopmental conditions has come from rodent studies from our group and others.[15–17] The consensus is that the MIA model mimics many features relevant to schizophrenia and autism in humans.[18,19]

Although not a genetic model, gene and protein expression differences have consistently been reported in MIA rodent models, including in the adult frontal cortex[20] and the fetal whole brain.[17,21,22] In an MIA model elicited by exposing pregnant rodents to the viral analog Polyinosinic:polycytidylic acid (PolyI:C), interleukin 6 (IL6)-induced inflammation has been shown to exert an epigenetic influence by regulating the methyltransferase gene.[23] Consistent with this, IL6 is been shown to alter global DNA methylation in diseases such as oral cancer;[24] however, it is unknown whether this occurs in the brain of offspring exposed to MIA.

One function of DNA methylation is to repress the transcription of repetitive retroviral elements in the genome including long interspersed element 1 (LINE1) or intracisternal A-particles (IAPs). IAPs are endogenous retroviral sequences, an important class of transposable elements that 'jump' within the genome inducing genomic mutations and cell transformation. LINE1 elements are retrotransposons - a subclass of transposons that also modulate gene expression, especially in the developing brain.[25] However, whether prenatal exposure to maternal inflammation alters methylation state of these repetitive elements has not been directly examined.

An important target for epigenetic modification in models of neurodevelopment is the Methyl CpG-binding protein2 (*Mecp2*).[26] MECP2 is involved in the timely activation and repression of gene expression. It has an important role in control of methylation[27] and has a regulatory role in neuronal transcription of LINE1.[25] It is strongly associated with neurodevelopmental disorders such as Rett syndrome,[28] autism,[29] schizophrenia and neural tube

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defects.[30,31] To date however, whether *Mecp2* is epigenetically altered in the MIA model has not been investigated.

In this study we tested the hypothesis that prenatal exposure to MIA in the mouse results in global methylation differences in the brain and specifically alters DNA methylation in the promoter of *Mecp2*. We elected to study the striatum because of its established association with schizophrenia[32] and autism,[33] and the hypothalamus that has an important influence on the limbic system and is thought to have a critical role in the onset of prodromal features of schizophrenia during the pubertal/postpubertal period.[34,35]

MATERIALS AND METHODS

Animal model of neurodevelopmental disorders

Female and male C57BL/6N mice were bred and mated in the Laboratory Animal Unit, The University of Hong Kong. The holding space had a 12:12 h normal light–dark cycle (lights off at 1900 hours), and temperature and humidity-controlled (21 ± 1 °C, 55 ± 5%) animal vivarium. Pregnant females were not disturbed, except for weekly cage-cleaning. Animals were maintained under *ad libitum* food and water supplied by the Laboratory Animal Unit. All experiments were performed in accordance with the relevant institutional and national guidelines and regulations approved by the Committee on the Use of Live Animals in Teaching and Research at The University of Hong Kong, and every effort was made to minimize the number of animals used and their suffering.

The MIA model was prepared following the procedures previously reported in our laboratory and others.[18,36] Potassium salt of Poly I:C was obtained from Sigma-Aldrich (Gillingham, UK) and dissolved in saline. A dose of 5 mg kg^{-1} in an injection volume of 5 ml kg^{-1}, prepared on the day of injection, was administered to pregnant dams on gestation day 9 via the tail vein under gentle physical restraint. The resulting offsprings were weaned and sexed at postnatal day 21. The pups were weighed and littermates of the same sex were caged three to four per cage.

At 6 weeks, mice were killed by cervical dislocation, and their brains were removed quickly. The hypothalamus and striatum were collected in 1.5-ml tubes using careful dissection methods referring the Allen Mouse Brain Atlas[37] and frozen in liquid nitrogen for storage. Ten to fifty milligrams of brain tissue were homogenized on ice in a new reaction tube with freshly made and autoclaved lysis buffer (10 mM Tris-HCl, 0.1 M EDTA, 0.5% sodium dodecyl sulfate) using a DNase-free, sterile plastic pestle. Samples were lysed by incubation with 300 ml of lysis buffer and 3.3 µl Proteinase K (18.5 mg ml^{-1}) for 16 h in a water bath at 50 °C. Samples were mixed thoroughly to break up any unlysed tissue and 1.65 µl Proteinase K (18.5 mg ml^{-1}) was added to the sample followed by 1-h incubation at 50 °C. Proteinase K was inactivated by incubating samples at 65 °C for 30 min.

Lysates were transferred to 2 ml eppendorf tubes and extracted with 300 ml of phenol–chloroform–isoamyl alcohol (25:24:1) buffered to pH 7.5 by inverting 20 times and centrifuging at 13 000 r.p.m. for 15 min. The aqueous layer was extracted twice with 300 ml of chloroform–isoamyl alcohol (24:1) in a new reaction tube, by inverting 20 times and centrifuging at 13 000 r.p.m. for 15 min. The aqueous layer was incubated with 75 µl of 10 M ammonium acetate (NH_4Ac) and 600 ml ice-cold 100% ethanol in a new reaction tube at − 20 °C for 1 h. The supernatant was removed after centrifugation at 13 000 r.p.m. for 15 min and the pellet was washed with 1 ml of 70% ethanol. DNA was pelleted by centrifuging at 13 000 r.p.m. for 10 min. The pellets were left to air dry for 1 h and resuspended in 100 µl of TE buffer. A NanoDrop ND-1000 was used to quantify the DNA samples, and the quality and size of samples were checked using a 1% agarose gel using gel electrophoresis.

Sodium bisulfite conversion

In all, 500 ng of genomic DNA from each sample was treated with sodium bisulfite in duplicate, using the EZ 96-DNA methylation kit (Zymo Research, Orange, CA, USA), following the manufacturer's standard protocol. The kit exploits the three-step reaction that takes place between cytosine and sodium bisulfite where unmethylated cytosine is converted to uracil. The DNA was resuspended in 30 µl of distilled water and stored at − 20 °C until further use.

LINE1 and IAP assay

We measured highly repetitive element methylation, blinded to epidemiological exposure condition. Previously reported sequences of forward and reverse primers of LINE1 and IAP[38,39] Sigma-Aldrich were used for analysis with the Sequenom EpiTYPER (San Diego, CA, USA), using universally methylated DNA as a methylated reference (EMD Millipore Corporation, Darmstadt, Germany), and an unmethylated DNA as negative control. The LINE1 and IAP element assays were designed to cover more than 600 000 genomic locations, which share a consensus sequence.[40]

Mecp2 promoter DNA methylation assay

Mecp2 promoter DNA methylation was quantified using the Sequenom EpiTYPER platform, and primers for the target region were designed using EpiDesigner (www.epidesigner.com, Sequenom). Six CpG sites were quantified in an amplicon spanning 282 bp in the promoter and the first exon (Please see Supplementary Table 1 for primer details). All EpiTYPER assays were tested using a standard curve with methylated and unmethylated DNA. Genomic DNA was isolated and 1 µg DNA was bisulfite-treated as described above using the EZ DNA methylation kit (Zymo Research).

Statistical analysis

Global DNA methylation was quantified in the two brain regions of interest (the hypothalamus and striatum) from 22 Poly I:C-exposed and 17 saline-exposed 6-week-old mice using the Sequenom EpiTYPER platform. The sample characteristics are shown in detail in Table 1. The samples were handled together to reduce experimental/handling bias at all stages of experiments. In all group comparisons each animal was treated as an individual subject. Both LINE1 and IAP assays measure a proxy of global DNA methylation, and Pearson correlation testing was used to identify any correlation between assays. A general linear model (GLM) was used to identify any main effect of group or sex or any interaction with unpaired *t*-tests to explore the data *post hoc*. Grubb's test using extreme studentized deviate method was used to detect outliers with an alpha cutoff >0.01. The criterion for statistical significance was set as $P<0.05$ and analyses were conducted using R (http://www.R-project.org/) and SPSS. The effect sizes reported are the absolute change (delta) in DNA methylation.

RESULTS

Altered global methylation in the adolescent brain following prenatal immune challenge

Quantitative DNA methylation was measured across LINE1 elements to give a proxy of global DNA methylation across ~600 000 repeats in the mouse genome.[41,42]

LINE1 methylation in the hypothalamus

There was a significant main effect of treatment ($F(1, 36)=5.72$, $\beta=-0.516$, $P=0.022$), with no effect of sex ($F(1, 36)=1.27$, $\beta=0.248$, $P=0.266$) on the mean LINE1 methylation, and 12.18% of variance was explained with the GLM used. This resulted from significant hypomethylation in the hypothalamus of the Poly I:C group (69.66%, s.d. = 0.62) compared with the saline group (70.19%, s.d. = 0.72; $t=2.44$, $P=0.019$, delta methylation = 0.53; see Table 2), especially in females ($t=3.04$, $P=0.005$, Poly I:C group mean 69.45, s.d. = 0.59, saline group mean 70.26, s.d. = 0.71, delta methylation = 0.81).

Table 1. Animal numbers

Group	*Poly I:C*			*Saline*	
Litter 6 weeks	*M1*	*M2*	*M3*	*M4*	*M5*
Sex (male:female)	3:3	3:6	2:5	4:5	3:5
Total		22		17	

Poly I:C group (litter M1, M2 and M3) and saline group (M4 and M5) with the number of males and females in each litter. Animals used in the experiment were, as far as possible, kept free from any interventions that could further affect their epigenetic status.

Table 2. PolyI:C and saline group comparison results of different assays

	LINE1			*IAP*			*Mecp2*		
	Mean	*s.d.*	P-*value*	*Mean*	*s.d.*	P-*value*	*Mean*	*s.d.*	P-*value*
Hypothalamus									
	0.696	0.006					0.265	0.067	
PolyI:C	F 0.694	0.005		0.935	0.014		F 0.290	0.037	
			0.019*						0.002**
	M 0.700	0.005					M 0.148	0.042	
			F 0.005**			0.47			F 0.000***
	0.701	0.007					0.346	0.059	
			M 0.885						M 0.202
Saline	F 0.702	0.007		0.931	0.012		F 0.362	0.046	
	M 0.700	0.007					M 0.266	0.068	
Striatum									
	0.707	0.011		0.930	0.050		0.295	0.179	
PolyI:C			0.29			0.85			0.37
Saline	0.704	0.010		0.936	0.066		0.345	0.121	

Abbreviations: F, Female; IAP, intracisternal A particle; LINE1, long interspersed elements; M, Male; Mecp2, Methyl CpG Binding Protein2. Mean methylation of all the markers for each assay. *$P < 0.05$, **$P < 0.005$ and ***$P < 0.0005$.

The first CpG in the LINE1 element in the hypothalamus was differentially methylated in adolescent stage after PolyI:C administration in prenatal life, main effect of Group ($F(1, 36) = 5.77$, $\beta = -1.503$, $P = 0.021$). In all, 9.08% of the variance was explained by the GLM used. There was no significant main effect of sex ($F(1, 36) = 0.08$, $\beta = -0.183$, $P = 0.775$); however, there was a significant Group x Sex interaction ($F(1, 35) = 5.54$, $\beta = 2.846$, $P = 0.024$) and the GLM explained 19.27% of variance. This was explained by significant hypomethylation in the PolyI:C group ($t = 2.42$, delta methylation = 1.49, $P = 0.02$; PolyI:C group mean = 27.68%, saline group = 29.17%, s.d. = 2.03). Again, differences were most pronounced in females ($t = 4.11$, delta methylation = 2.62, $P < 0.001$).

LINE1 methylation in the striatum

There was no significant main effect of group, or sex, or any Group x Sex interaction on the mean LINE1 methylation in the striatum (PolyI:C group mean = 70.79, s.d. = 1.15 and saline group mean = 70.41, s.d. = 1.01).

IAP assay

IAP promoters were not differentially methylated in the hypothalamus or striatum in the PolyI:C and saline control groups, see Table 2. LINE1 and IAP assays for the mean global methylation were not correlated ($P = 0.298$, $r = -0.170$).

Mecp2 promoter methylation

The mean methylation of *Mecp2* in the polyI:C-exposed hypothalamus was lower than in saline groups (PolyI:C group = 26.57%, s.d. = 6.70; saline group = 34.63%, s.d. = 5.97). There was a significant effect of group and sex in the mean methylation of the *Mecp2* promoter region in the hypothalamus: ($F(1, 26) = 24.08$, $\beta = -7.937$, $P < 0.001$) and ($F(1, 26) = 34.49$, $\beta = 12.384$, $P < 0.001$), respectively. In total, 67.18% of variance in *Mecp2* methylation was explained by the GLM used. There was no significant Group x Sex interaction. *Post hoc* tests confirmed *Mecp2* promoter hypomethylation in the PolyIC group ($t = 3.39$, delta methylation = 8.05, $P = 0.003$) and in females ($t = 4.35$, delta methylation = 7.14, $P < 0.001$). Please see Figure 1 and Table 2.

Individual CpG sites 2 and 4 were significantly differentially methylated in each group ($F(1, 26) = 13.12$, $\beta = -9.870$, $P = 0.001$; $F(1, 26) = 11.71$, $\beta = -6.244$, $P = 0.002$, respectively) and sex ($F(1, 26) = 10.84$, $\beta = 11.294$, $P = 0.003$; $F(1, 26) = 13.08$, $\beta = 8.604$, $P = 0.001$, respectively). The mean methylation for CpG site 2; PolyI:C group = 20.35%, s.d. = 8.71, saline group = 30.33%, s.d. = 7.24 and CpG site 4; PolyI:C group mean = 30.58%, s.d. = 6.21, saline group = 36.91%, s.d. = 5.19. There was no significant Group x Sex interaction. A total of 15.63% of variance in CpG 2 methylation and 45.22% of variance in CpG 4 methylation were explained by the GLM used.

Mean methylation of *Mecp2* in polyI:C-exposed striatum was also lower than controls but this difference did not reach statistical significance (please see Figure 1 and Table 2).

DISCUSSION

This is the first study to directly examine the epigenetic consequences of prenatal exposure to MIA in the adolescent rodent brain. We found significant DNA hypomethylation in the hypothalamus and a similar trend in the striatum of offspring exposed to MIA compared with saline-exposed controls. In addition, the *Mecp2* gene promoter region was significantly hypomethylated in the hypothalamus of MIA-exposed group but not in the striatum. These effects were most pronounced in females.

LINE1 methylation

Approximately 50% of the mammalian genome comprises the retrotransposon-associated repeat sequences, with LINE1 elements and LTR elements (including IAP) accounting for 20%[40] and 10%, respectively.[43] LINEs are ~6-kb-long elements with an internal polymerase II promoter encoding two open reading frames and account for a great amount of somatic mosaicism.[44] LINE1 elements transpose through RNA intermediates, assembling with their own encoded proteins through reverse transcription. LINE1 is highly active and can create indels, new splice sites which fine-tune gene expression.[45] LINE1 transposons may also disrupt coding regions, and their epigenetic suppression by causing indels is vital during the early course of development.[46] Inappropriate suppression caused by altered DNA methylation across these elements may lead to adverse consequences in the adult life.[47]

Consistent with this role in development, LINE1 has been reported to be differentially methylated and highly susceptible to retrotransposition in neurodevelopmental disorders.[25] For example, hyperactive retrotransposition and higher copy number of LINE1 have been reported in schizophrenia and observed in the

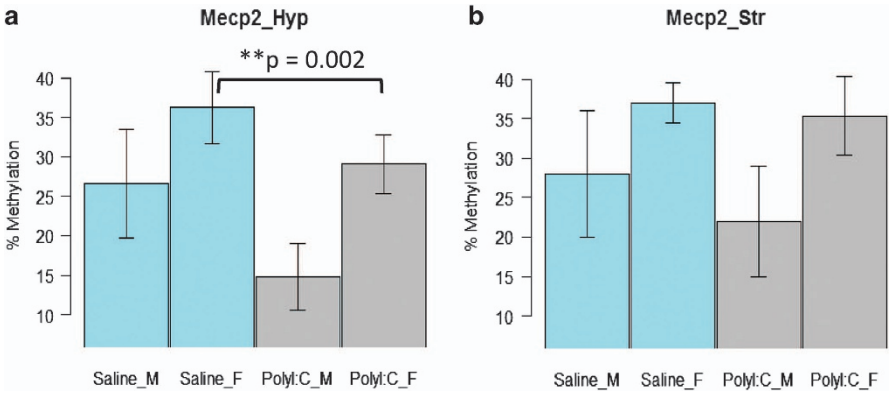

Figure 1. Hypomethylated *Mecp2* promoter in the hypothalamus of PolyI:C-exposed offspring. *y* axis shows methylation in percentage with error bar as s.e.m., histograms represent saline- and polyI:C-treated male and female groups. (**a**) *Mecp2* promoter methylation in the hypothalamus of polyI:C- and saline-exposed male and female groups. (***P*-value is calculated from a stratified *t*-test between saline- and polyI:C-affected females). (**b**) *Mecp2* promoter methylation in the striatum of polyI:C and saline-exposed male and female groups.

polyI:C MIA model.[48] Our observation of LINE1 hypomethylation in the same MIA model would be expected to increase retrotransposition and may provide one possible path linking inflammation with the increased risk of schizophrenia.

Altered DNA methylation as a potential mediator of environmental risk for neurodevelopmental disorders fits with evidence from other systems. For example, prenatal exposure to the antiepilepsy drug valproate is associated with increased risk of autism spectrum disorders in the offspring,[49] and this drug has been recognized to cause widespread epigenetic reprogramming especially DNA hypomethylation.[50]

IAP methylation

IAPs are autonomous retrotransposon elements with long terminal repeats and all the necessary transcriptional regulatory elements. These class-II endogenous retroviruses transpose through retroviral mechanisms in the cisternae of the endoplasmic reticulum.[51] IAPs add a significant amount of genetic variabilities to the genome with differential tissue-specific expression and are capable of interfering gene function in the brain.[52,53] The complexity and the size of IAP make it less common and less likely to occupy the vital regions in the genome. Thus, IAP assays target completely different elements than LINE1, with a lower representation over the genome,[54] which may account for the sparsity of findings using this assay in our study, and the lack of evidence linking IAP alterations with neurodevelopmental disorders.

Mecp2 methylation

MECP2 has a well-established role in Rett's syndrome but has also been implicated, by our group and others, in idiopathic neurodevelopmental disorders such as autism and schizophrenia.[55,56] MECP2 is an abundant neuronal protein that indirectly mediates gene transcription by recruiting transcription factor cAMP responsive element-binding protein 1, depending on the methylation status of the DNA.[27] This action alters the expression of many genes in the hypothalamus[57] and beyond, including LINE1. Aberrant promoter methylation of *MECP2* itself is thought to contribute to a defective biological network and potentially to the etiology of neurodevelopmental disorders.[58]

Here we report a significant association between MIA and *Mecp2* promoter hypomethylation. The polyI:C used in this MIA model is known to cause an increase in IL6.[59] MECP2 in turn may suppress *IL6* expression;[60] therefore, we speculate that hypomethylation, leading to a higher level of MECP2, may be involved in silencing genes that are activated by the immune response. Changes in *Mecp2* could theoretically influence the expression of other developmental genes previously reported to be altered in the MIA model, for example, brain-derived neurotrophic factor[61] and Reelin, Glutamate decarboxylase 67[62] and cAMP response element-binding protein;[63] histone deacetylases and DNA (cytosine-5)-methyltransferase 1.[26,64] A theoretical construct showing a functional protein association network potentially influenced by MECP2 is shown in Supplementary Information and Figure 1.[65]

More specifically, in terms of the dopamine system, closely linked to disorders such as schizophrenia, we speculate that removing a methylation 'brake' on *Mecp2* gene expression would promote or even overdevelop dopaminergic neurons. This is because the converse - low levels of MECP2 in dopamine neurons - results in fewer dendrites, less membrane surface area and even a compromised nigrostriatal pathway.[66]

PolyIC caused greater LINE1 hypomethylation in the hypothalamus of female offspring; however, although the *Mecp2* promoter was more hypomethylated in females in both polyI:C- and saline-exposed groups, there was no significant Sex x Group interaction. Although neurodevelopmental disorders such as autism are generally more prevalent in males than females, this is not the case for schizophrenia,[67] and Rett syndrome is predominantly diagnosed in females.[68] Moreover, consistent with our results, genome-wide methylation studies of schizophrenia and other psychiatric conditions have reported global hypomethylation in females.[69,70] This excess of epigenetic modification in the female sex may also be relevant to the suggestion that environmental exposures are relatively more important in females with neurodevelopmental disorders.[71]

Regional methylation changes

Hypomethylation of *Mecp2* and LINE1 in an adolescent offspring exposed to MIA was most pronounced in the hypothalamus. Hypothalamus–pituitary–adrenal axis developmental changes, and especially hypothalamic dopamine systems, are strongly implicated in the onset of schizophrenia.[35,72] Consistent with this evidence, MECP2 has been found to mediate dopaminergic precursor differentiation in the ventral midbrain especially in the developing hypothalamus via *Dlk1*.[73] Similarly, aminergic neurotransmitter concentration is regulated by MECP2 through rate-limiting enzymes involved in synthesis and degradation, which may contribute to phenotypes relevant to schizophrenia and autism.[74]

The direction of *Mecp2* methylation differences in the striatum of MIA exposed mice relative to controls was similar to those observed in the hypothalamus; however, group differences did not reach statistical significance. We also observed a main effect of sex - females were generally undermethylated relative to males in

both PolyI:C and control groups in both regions of interest. Thus, whereas low power may have limited any group differences in methylation in the striatum, it is also possible that lower methylation in females of both groups may have obscured group differences in methylation in the striatum.

Advantages and limitations of the study

An organism's phenotype is produced by its genotype's interaction with the environment and thus epigenetic changes influence gene expression, DNA repair and recombination. Under the influence of these epigenetic mechanisms, genetically identical cells, tissues and organs show variation and become unique during the course of development.[75] However, investigating epigenetic etiological mechanisms in the brain, and understanding the effect of epigenetic modifications resulting from the prenatal assaults in humans, is extremely challenging. This is partly because studies are indirect, relying upon peripheral blood, buccal swabs and other tissues.

Therefore, animal models can help to better control the environmental exposures and homogeneity of the sample. Moreover, the animal model allows DNA isolation from specific regions of the brain. This is important because patterns of DNA methylation are region- and tissue-specific,[76,77] and peripheral tissue samples collected in humans are unlikely to reflect what is happening in the brain.[78] However, although animal models allow us to sample brain, there are still technical constraints and it must be acknowledged that assays such as LINE1[45] and IAP provide only a proxy of global methylation.[6]

We also accept that the current study targeted restricted regions of the brain; therefore, we cannot say whether our findings are truly region-specific or more generalized. Even though carefully dissected out, DNA was isolated from tissue comprising a mix of cells with possibly different epigenetic profiles. Assays of single cell or minimal numbers of isolated cells could have improved cell type accuracy but also have restricted generalization. Furthermore, we used LINE1 and IAP assays only to obtain a proxy of global DNA methylation. These assays cannot reveal the individual genes affected, nor tell us whether the differences observed have functional implications. Future work will need to be conducted to ascertain the functionality of these methylation changes (especially the significant, but very small, group difference in LINE1 DNA methylation in the hypothalamus).

Finally, we acknowledge that alternative design would be to consider each litter as a single experimental unit with the assumption that the exposure of fetuses within one litter is identical. However, this assumption may not always hold as each fetus in the mouse uterus has been shown to have a distinct blood perfusion pattern;[79] therefore, exposures may not necessarily be identical. On balance, we wished to optimize genetic similarity so that the focus would be on environmental exposure. We therefore elected to treat each an offspring as a 'subject', and enter data from each littermate separately into the analysis.

Summary

This study examined epigenetic differences in the hypothalamus and striatum of the adolescent brain following exposure to MIA in prenatal life, a risk factor for schizophrenia and related neurodevelopmental conditions. We observed differences in both global methylation and *Mecp2* promoter methylation that may have relevance to neurodevelopmental disorders. In particular, hypomethylation of the *Mecp2* promoter may have widespread repercussions on gene expression and deserves further exploration as a possible biomarker for environmental modifications linked to prenatal assault. Further exploration of epigenetic mechanisms operating in neurodevelopmental conditions is warranted because these gene modifications are potentially modifiable and this approach may open new avenues for treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)